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Mating pattern in pure hybrid populations of water frogs, *Rana* kl. *esculenta* (Anura, Ranidae)*

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The existence of pure hybrid populations is one of the most interesting phenomena within the *Rana* kl. *esculenta* synklepton. The aim of our investigations, carried out on two *esculenta* populations, was to clarify whether particular genotypes are favoured during mating. A comparison between the theoretical frequencies of every mating combination (diploid male × diploid female, diploid male × triploid female, triploid male × diploid female, triploid male × triploid female), calculated on the basis of the population structure, and the observed frequencies yielded no significant differences. This indicates that diploid and triploid individuals have equal chances to mate. Triploid females probably play a secondary role in the population structure as their frequencies amounted to only 5.5 % in the population A and to 6.6 % in the population B. Diploid males were found in a relatively high proportion in the population A (19.5 %), while they amounted to only 6.6 % of individuals in the population B. While in population A the mating combinations, diploid male × diploid female and triploid male × diploid female, had nearly the same frequencies, in population B most matings occurred between triploid males and diploid females.

Moreover, we found no clear evidence for mating choice in relation to body size.

PROBLEM

In Central Europe the hybridogenetic edible frog, *Rana* kl. *esculenta*, mainly lives in populations together with only one of its two parental species, either *Rana lessonae* or *Rana ridibunda*. For its reproduction *esculenta* is fundamentally dependent on these parental forms. Due to their hybridogenetic gamete producing system the hybrids mainly give rise to pure parental gametes, while genetic recombination and introgression occur at a very low level (UZZELL & BERGER, 1975; TUNNER & DOBROWSKY, 1976; UZZELL et al., 1977; GÜNTHER, 1973). Surprisingly, pure hybrid populations exist in several parts of Europe, for example in Germany, Poland, Sweden and probably Denmark (see GUNTHER, 1973, 1974, 1975, 1990; EBENDAL, 1979, EIKHORST, 1984; BERGER, 1988).

In the pure *esculenta* populations it could be shown that a certain number of frogs were triploid (GÜNTHER, 1975). Moreover, by means of morphological, serological and enzymological studies it became evident that these hybrids had two genetic compositions: one with two *lessonae* genomes and one *ridibunda* genome (LLR) and a second with one

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lessonae genome and two *ridibunda* genomes (LRR). The latter are very rare in most hybrid populations in Germany (GÜNTHER, UZZELL & BERGER, 1979; GÜNTHER, 1983; BERGER & GÜNTHER, 1988).

While the reproductive system of mixed populations has been more or less well elucidated, the mechanisms for the maintenance of pure hybrid populations have not yet been clarified in all their details. Considering the joint occurrence of six different genotypes (LR males, LR females, LLR males, LLR females, LRR males, LRR females) theoretically nine different mating combinations may occur. The aim of our study was to clarify whether mating occurs by chance or whether mating preferences between certain genotypes exist. In this context relations between the observed mating frequencies of different genotypes and the genotypic structure of the population are discussed.

Besides, we investigated the significance of body size of water frog males and females for mating choice.

MATERIAL AND METHODS

The study was performed on individuals from two populations: population A was found in an eutrophic garden pool in Berlin, population B lives in a small pond in a meadow near Boltenhagen, at the shore of the Baltic Sea. In both populations only *esculenta* phenotypes were found. In the population A 15 pairs were captured on May 18th, 1988 and 22 additional pairs on May 26th of the same year. In the population B 18 pairs were caught on May 20th, 1975. All pairs were in amplexus.

Besides, in both populations non-paired individuals were collected at random at the same dates (population A: 16 males and 38 females, population B: 16 males and 10 females). Ploidy was determined indirectly by means of cytomorphological parameters (mean length, width and surface of erythrocytes; see GÜNTHER, 1977).

For all individuals the snout-vent-length (s.v.l.), the length of the first toe (d.p.l.) of the metatarsal tubercle (c.int.l) and of the tibia (t.l.), as well as the distance between the nostril and the caudal eye edge (d.n.e.) and the head width (h.w.) were measured. The ratios, s.v.l./c.int.l., d.p.l./c.int.l., t.l./int.l., h.w./c.int.l. and d n.e./c.int.l., constituted the basis for determining genotype together with the cytomorphological parameters

In the analysis of the data the Chi-square test, to test goodness of fit between observed (f_o) and expected frequencies (f_e), was utilized

GENOTYPIC STRUCTURE

The results of the ploidy analysis and sex ratios in populations A an B are summarized in Table I. In both populations the relation of males to females deviates from the expected 1:1 ratio. However, these deviations are not significant (population A: $\chi^2 = 3.78$, d.f. = 1, $p > 0.05$; population B: $\chi^2 = 0.58$, d.f. = 1, $p > 0.05$).

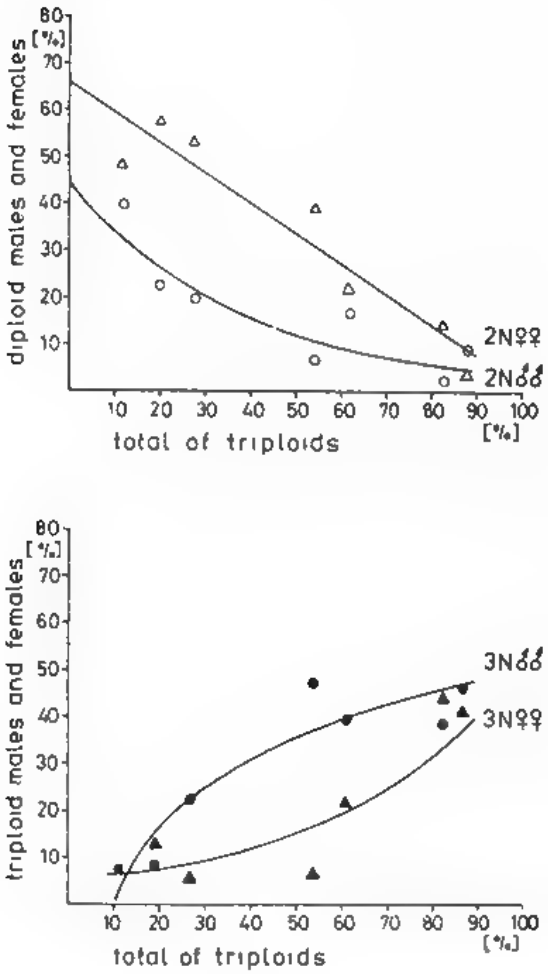

Fig. 1. – Proportion of observed 2N males, 2N males and 3N females in relation to the total of triploids (males + females) in pure *esculenta* populations Data were taken partly from: GÜNTHER, 1975, BERGER, 1988; BERGER & GÜNTHER, 1988. The functions are empirically fitted to the data

Table I. – Proportion of different genotypes in populations A and B (m, males; f, females; 2N, diploid; 3N, triploid).

Sample	n	Proportion of genotypes						Sex ratio
		2N	3N	2N m	2N f	3N m	3N f	m: f
A	128	93 (72.7 %)	35 (27.3 %)	25 (19.5 %)	68 (53.1 %)	28 (21.9 %)	7 (5.5 %)	1. 1.42
B	61	28 (45 9 %)	33 (54.1 %)	4 (6 6 %)	24 (39.3 %)	29 (47.5 %)	4 (6.6 %)	1· 0 82

The proportion of triploid individuals in populations A and B are significantly different ($\chi^2 = 12.83$, d.f. = 1, $p > 0.001$). In the population A only 27.3 % triploid individuals were found, while in the population B their frequency was 54.1 %.

While the proportion of triploid females in both populations was quite similar (5.5 and 6.6 %), the high triploid rate in the population B can be attributed to the relatively large number of triploid males (47.5 %).

As fig. 1 shows, in pure *esculenta* populations the proportion of triploid individuals can be due mainly to the high proportion of triploid males. In most *esculenta* populations examined up to now, there exists an excess of males among triploid individuals. Only when

Table II. Morphological parameters of diploid (2N) and triploid (3N) *Rana* kl. *esculenta* from populations A and B in comparison to the same parameters of central European *Rana lessonae* and *Rana ridibunda*. n, number of individuals; s.v.l., snout-vent-length; c.int.l., callus internus length; d.p.l., digitus primus length; t.l., tibia length; h.w., head width; d.n e., eye-nostril distance.

Genotype	n	Ratio sv.l./ c.int.l.	d.p.l./ c.int.l.	t.l./ c.int.l.	h.w./ c.int.l.	d.n.e / c.int.l.
Rana kl. *esculenta*						
Sample A						
2N males	21	13 6-17.5 (15 8 ± 1.10)	1.8-2.5 (2.2 ± 0.19)	6.9-9.0 (7.9 ± 0.60)	5.3-6.7 (5.9 ± 0 41)	2.6-3.5 (3.0 ± 0.22)
2N females	48	14.4-18.6 (16 0 ± 1.13)	1.9-2.7 (2.2 ± 0.18)	6.6-9.4 (7.7 ± 0.60)	4.5-6 8 (5.7 ± 0.52)	2.5-3.5 (2.9 ± 0.24)
3N males	21	13.7-16 5 (15.0 ± 0.78)	1 6-2.2 (2.0 ± 0.16)	6.7-7.8 (7.3 ± 0.37)	5.0-6 1 (5.5 ± 0.26)	2 6-3 2 (2.9 ± 0.16)
3N females	5	15.3-16.1 (15.7 ± 0.35)	1 9-2 2 (2.0 ± 0.10)	7.1-7.8 (7.5 ± 0.24)	5.7-5.8 (5.8 ± 0.04)	2 9-3 0 (2.9 ± 0 04)
Sample B						
2N males	3	15.6-17.4 (16.3 ± 1.0)	2.2-2 4 (2.3 ± 0.10)	7 6-8.3 (7.9 ± 0 35)	5 5-5.8 (5.6 ± 0.18)	2.8-3.0 (2.9 ± 0.1)
2N females	24	15.4-20.2 (17.8 ± 1.24)	2.2-2.9 (2.6 ± 0.19)	7.3-9.3 (8.4 ± 0 52)	5.7-7.2 (6.2 ± 0.37)	2.6-3.6 (3.1 ± 0.22)
3N males	28	14.3-18.0 (16.1 ± 0.94)	2.0-2.4 (2.2 ± 0.13)	6.9-8.3 (7.7 ± 0 36)	5.0-6.4 (5 6 ± 0.32)	2.4-3.4 (3.0 ± 0.20)
3N females	4	16.3-19.5 (17.4 ± 1.52)	2.2-2.5 (3.3 ± 0.20)	7.7-9.0 (8.1 ± 0.60)	5.6-6.5 (6.0 ± 0.36)	2.8-3.5 (3.1 ± 0.28)
Rana lessonae	19	10.0-14 3 (12.3 ± 1.17)	1 3-1.7 (1.5 ± 0.10)	5.1-6.7 (5.9 ± 0.41)	3.5-5 3 (4.5 ± 0 45)	2.2-2.8 (2.6 ± 0.18)
Rana ridibunda	47	17.4-25.4 (20.7 ± 1.93)	2.3-3.9 (3.0 ± 0.36)	9 2-14.2 (11.1 ± 1.06)	6.3-9 3 (7.6 ± 0.80)	3 1-4.7 (3 8 ± 0.39)

the proportion of triploids reaches about 80 % does the ratio 3N males / 3N females seem to become more or less balanced again. Moreover, it is noteworthy that in all populations examined up to now, there was a clear excess of females among the diploid individuals

Morphological parameters of the investigated individuals are given in Table II. In both populations the values of these parameters were slightly lower in the triploid than in the diploid individuals. Compared with the parental species the values of the diploid as well as the triploid individuals were more similar to those of *Rana lessonae*. This fact, the frequency-distribution of the morphological parameter and cluster-analyses, carried out on the basis of these ratios, lead to the assumption, that with one possible exception all triploid individuals possess one *ridibunda* and two *lessonae* chromosome sets (LLR genotype) (PLÖTNER, unpublished). The ratios of one female (No. 45) from population B showed values that are *ridibunda* specific. However, the metatarsal tubercle exhibited a shape typical for diploid *Rana* kl. *esculenta*. Possibly this female was of the genotypic composition LRR.

MATING FREQUENCIES OF INDIVIDUAL GENOTYPES

Due to the existence of four different genotypes: 2N [LR] males, 2N [LR] females, 3N [LLR] males and 3N [LLR] females in both populations, the following four mating combinations can occur:

1. 2N [LR] male × 2N [LR] female
2. 2N [LR] male × 3N [LLR] female
3. 3N [LLR] male × 2N [LR] female
4. 3N [LLR] male × 3N [LRR] female

In order to clarify the question whether certain mating combinations occur more frequently than others, the expected frequencies (f_e) of individual combinations must first be calculated on the basis of the genotypic structure of each population. As the proportion of each genotype was estimated from a random sample, f_e only represents an approximate value. f_e was calculated according to the formula:

$$f_e = \frac{n_{mj}}{n_m} \quad \frac{n_{fj}}{n_f} \quad n_p$$ where

n_{mj} = number of male genotypes of the corresponding combination,
n_{fj} = number of female genotypes of the corresponding combination,
n_m = total of males in the mated and unmated subsample,
n_f = total of females in the mated and unmated subsample,
n_p = total of pairs captured in the population.

Table III shows the expected and observed frequencies of all mating combinations The differences between the observed and expected frequencies are not statistically significant, neither in population A ($\chi^2 = 0.94$, d.f $= 3$, $p > 0.05$) nor in population B ($\chi^2 = 3.85$, d f. $= 3$, $p > 0.05$).

Table III. – Observed (f_o) and expected (f_e) frequencies of all possible mating combinations in populations A and B (m, male; f, female, 2N, diploid; 3N, triploid).

Combination m × f	Population A Observed frequency f_o	n_p = 37 Expected frequency f_e	Population B Observed frequency f_o	n_p = 18 Expected frequency f_e
2N × 2N	18	15.825	1	1.868
2N × 3N	1	1 628	0	0 311
3N × 2N	17	17.723	17	13 558
3N × 3N	1	1 824	0	2.259

In order to test whether the carriers of certain genotypes exhibit mating preferences, the frequencies of genotypes in paired and single adults were compared (Table IV). While in population A no significant deviations were found between the observed and the expected frequencies ($\chi^2 = 7.46$, d.f. = 3, $p > 0.05$), in population B these differences were significant at a 5 % level ($\chi^2 = 10.21$, d.f. = 3). This latter fact depends mainly on a greater number of observed 2N mated females than was theoretically expected. However, as the sample size in population B was relatively small, this result should be viewed cautiously. It can therefore be concluded that, in principle, all the genotypes possess equal mating chances.

Table IV. – Observed (f_o) and expected (f_e) frequencies for mated and unmated individuals of different genotypes in samples A and B (2N, diploid; 3N, triploid).

Genotype	Sample A				Sample B			
	Mated individuals		Unmated individuals		Mated individuals		Unmated individuals	
	f_o	f_e	f_o	f_e	f_o	f_e	f_o	f_e
2N male	19	14.45	6	10.55	1	2 36	3	1.64
2N female	35	39.31	33	28.69	18	14.16	6	9 84
3N male	18	16 19	10	11 81	17	17.11	12	11 89
3N female	2	4.05	5	2.95	0	2.36	4	1.64

In *esculenta* populations, LR males normally form haploid R gametes while LR females can produce haploid R as well as diploid LR gametes Triploid individuals of LLR genotype mainly form gametes with one *lessonae* genome and triploid ones of LRR genotype mainly form gametes with one *ridibunda* genome (see GÜNTHER, UZZELL & BERGER, 1979; GÜNTHER, 1983; BERGER & GÜNTHER, 1988).

The hypothesis sustained up to now that the structure and stability of *esculenta* populations is based mainly on crosses between triploid males and diploid females (see GÜNTHER, UZZELL & BERGER, 1979; GÜNTHER, 1988) seems to be valid only for such populations in which the proportion of triploids lies between 30 and 70 %. From such crosses diploid LR and triploid LLR individuals can originate. In populations consisting

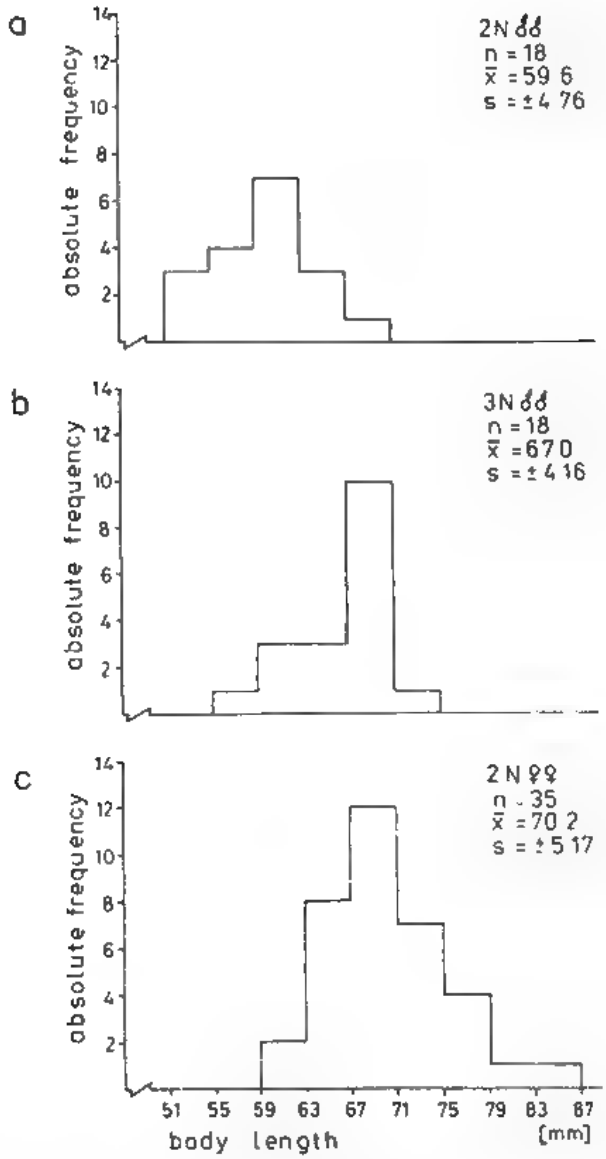

Fig. 2. Distribution of body length in members of pairs captured in amplexus in population A
a: 18 diploid males (combination 2N male × 2N female and 2N male × 3N females);
b: 18 triploid males (combination 3N male × 2N female and 3N male × 3N female);
c: 35 diploid females (combination 2N male × 2N female and 3N male × 2N female)

mainly of diploid individuals, most crosses should correspond to the combination 2N male × 2N female, while in those populations with a high proportion of triploids, the combination 3N male × 3N female should prevail (see fig. 1).

In most *esculenta* populations from Germany, the majority of triploid individuals are of LLR genotype and form gametes with one *lessonae* genome (see GÜNTHER, UZZELL & BERGER, 1979, GÜNTHER, 1983; BERGER & GÜNTHER, 1988). From crosses between two

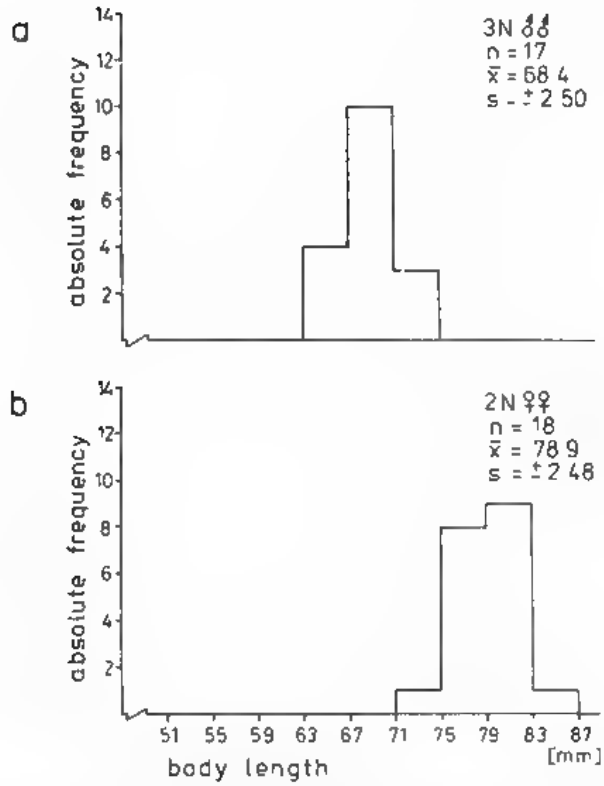

Fig. 3. Distribution of body length in members of pairs captured in amplexus in population B. a: 17 triploid males (combination 3N male × 2N female); b: 18 diploid females (combination 2N male × 2N female and 3 male × 2N female).

LLR individuals mainly *lessonae* (LL) genotypes result. It is known that these, just as the RR genotypes from LR × LR crosses, do not survive in *esculenta* populations. It follows that in *esculenta* populations with an increasing proportion of triploid individuals, either the rate of reproduction decreases and the population reaches an "end stage", or the LLR females must form, besides haploid L gametes, a certain number of diploid LR ones (see GÜNTHER, UZZELL & BERGER, 1979; GÜNTHER, 1983).

BODY SIZE OF MATING PARTNERS

In the mean, females in population A were 11 % and in population B, about 14 % larger than their male partners. The greatest difference (31 %) between the body length of a female (74 mm) and that of its male (51 mm) partner was found in population A in a pair of the 2N male × 2N female combination. The reverse situation also appeared in population A, in a combination 3N male × 2N female. Here the body length of the male

was 70 mm, that of the female, 62 mm; this corresponds to a difference of 11 % in favour of the male

Among the 55 pairs captured in amplexus the male was larger than the female in only four pairs (7.3 %) although, according to the distribution of body length (fig. 2a-c, 3a and b) at least in the population A, a higher proportion could have been possible. While in population B all the diploid females of the pairs were larger than the triploid males, in population A there was an overlap between the distribution of body length of the male and female partners. Although males seem to prefer larger to smaller females there is no clear evidence for significant size related mating preferences.

How mating choice takes place in European water frogs has not yet been clarified in detail.

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